

IN THE SPECIFICATION

At page 1, line 3 after the title of the invention, please **delete** the paragraph beginning “[t]his non-provisional application claims the benefit...”

Please **add** the following paragraph beginning at page 1, line 3 in place of the above-deleted paragraph:

The present application is a 371 national stage entry of PCT/EP03/12423, filed November 6, 2003, which international application claims the benefit of international application number PCT/US03/12901, filed April 25, 2003, which claims the benefit of U.S. Provisional Application Nos. 60/375,665, filed April 26, 2002, 60/375,834, filed April 26, 2002, 60/375,779, filed April 26, 2002, and 60/375,622, filed April 26, 2002, and which international application PCT/EP03/12423 also claims the benefit of PCT/US03/12943, filed April 25, 2003, which claims the benefit of U.S. Provisional Application Nos. 60/375,665, filed April 26, 2002, 60/375,834, filed April 26, 2002, 60/375,779, filed April 26, 2002, and 60/375,622, filed April 26, 2002, and which international application PCT/EP03/12423 also claims the benefit of international application PCT/US03/12926, April 25, 2003, which claims the benefit of U.S. Provisional Application Nos. 60/375,665, filed April 26, 2002, 60/375,834, filed April 26, 2002, 60/375,779, filed April 26, 2002, and 60/375,622, filed April 26, 2002. International applications PCT/EP03/12423, PCT/US03/12901, PCT/US03/12943, and PCT/US03/12926 are herein incorporated by reference in their entireties. U.S. Provisional Application Nos. 60/375,665, 60/375,834, 60/375,779, and 60/375,622 are also herein incorporated by reference in their entireties.

In the Summary of the Invention beginning at page 3, line 26, before the sentence “[t]hese and other embodiments of this invention are more fully described”, please **add** the following section entitled “Certain Embodiments”:

Certain Embodiments

Embodiment 1. A method comprising

- (a) identifying a non-nucleotide prototype compound;
- (b) substituting the prototype compound with a phosphonate-containing group to produce a candidate compound; and
- (c) determining the anti-HIV activity of the candidate compound.

Embodiment 2. A method comprising

- (a) selecting a non-nucleotide candidate compound containing at least one esterified carboxyl or esterified phosphonate-containing group; and
- (b) determining the intracellular persistence of the candidate compound or a esterolytic metabolite of the esterified carboxyl or phosphonate-containing group thereof.

Embodiment 3. The method of embodiment 1 wherein the tissue selectivity of the candidate compound and/or at least one of its intracellular depot metabolites is determined.

Embodiment 4. The method of embodiment, 1 wherein the intracellular residence time of said candidate compound and/or at least one of its intracellular depot metabolites is determined.

Embodiment 5. The method of embodiment 2 comprising additionally determining the activity of at least one of said metabolites against HIV protease.

Embodiment 6. The method of embodiment 2 wherein the metabolite is a carboxylic acid.

Embodiment 7. The method of embodiments 1 or 2 comprising determining the ability of the candidate to inhibit HIV.

Embodiment 8. The method of embodiment 1 wherein the prototype is already known to have therapeutic activity against HIV.

Embodiment 9. The method of embodiment 2 comprising selecting and determining the intracellular persistence of a plurality of candidate compounds.

Embodiment 10. The method of embodiments 1 or 2 wherein compounds which are not candidate compounds are tested in parallel together with at least one candidate compound.

Embodiment 11. The method of embodiment 2 comprising determining cleavage of one or more candidates by GS-7340 Ester Hydrolase.

Embodiment 12. The method of embodiments 1 or 2 wherein the candidate is an amino acid phosphonoamidate in which a carboxyl of the amino acid is esterified.

Embodiment 13. The method of embodiment 1 wherein the prototype compound is known to inhibit HIV protease, HIV integrase or HIV reverse transcriptase.

Embodiment 14. The method of embodiment 1 wherein the prototype compound is not known to be an analogue of a naturally occurring phosphate-containing enzyme substrate.

Embodiment 15. The method of embodiment 1 wherein the prototype compound is not a nucleoside.

Embodiment 16. The method of embodiment 1 wherein the prototype compound does not contain a nucleoside base.

Embodiment 17. The method of embodiment 1 wherein an intracellular depot metabolite is tested.

Embodiment 18. The method of embodiment 1 also comprising determining the resistance of HIV to the candidate compound and/or its intracellular depot metabolite.

Embodiment 19. The method of embodiment 1 comprising determining the tissue selectivity and/or intracellular residence time for a first candidate compound and/or its intracellular depot metabolite, preparing or selecting additional analogues of said first candidate compound, and determining the therapeutic activity of said additional analogues without determining tissue selectivity and/or intracellular residence time of said analogues.

Embodiment 20. The method of embodiment 1 comprising determining the safety and/or anti-HIV therapeutic activity of the candidate compound in *in vitro* cell culture, in enzyme assay, in animals or in humans.

Embodiment 21. The method of embodiment 1 wherein the prototype compound is a pharmaceutical product licensed by the US Food and Drug Administration.

Embodiment 22. The method of embodiment 1 wherein the prototype compound is one which is disclosed to have anti-HIV activity in a patent or published patent application on or before the filing date of this application.

Embodiment 23. The method of embodiment 1 comprising determining susceptibility to hydrolysis of the carboxyl or phosphonate esters by GS-7340 Ester Hydrolase, said Hydrolase characterized by being capable of being recovered from human PBMCs by a process comprising

- (a) lysing human PBMCs;
- (b) extracting the lysed cells with detergent;
- (c) separating the solids from supernatant and recovering the supernatant;
- (d) contacting the supernatant with an anion exchange medium;
- (e) eluting the Hydrolase from the anion exchange medium;

(f) contacting the eluate with a hydrophobic chromatographic medium; and

(g) eluting the Hydrolase from the hydrophobic chromatographic medium.

Embodiment 24. The method of embodiment 23 wherein the Hydrolase has a MW on gel filtration chromatography of about 70-100 kDa, has a pI of about 4.5-5.5 by chromatofocusing, is inhibited by 3,4 dichloroisocoumarin, binds to Butyl Sepharose HIC, binds to anion exchange medium Q15, and is capable of being recovered from human PBMCs.

Embodiment 25. The method of embodiment 2 wherein the intracellular residence time is determined as the half-life of at least one intracellular depot metabolite within a lymphoid tissue.

Embodiment 26. The method of embodiment 25 wherein the lymphoid tissue is PBMCs, helper cells, killer cells or lymph nodes.

Embodiment 27. The method of embodiment 1 wherein determining anti-HIV activity is by *in vitro* assay.

Embodiment 28. The method of embodiment 27 wherein the assay is conducted in an animal model or clinical trials.

Embodiment 29. The method of embodiments 1 or 2 comprising the additional steps of identifying a clinical trial compound from the final step, entering into clinical trials with said clinical trial compound, obtaining regulatory approval to market said clinical trial compound for the treatment of HIV, and selling said clinical trial compound after said regulatory approval.

Embodiment 30. The method of embodiment 29 wherein the clinical trial compound is not identical to the candidate compound.

Embodiment 31. The method of embodiment 2 wherein intracellular persistence was determined by clinical studies comprising determination of the amount and timing of dosing of the candidate compound.

Embodiment 32. The method of embodiment 2 wherein the metabolite is intracellularly sequestered in PBMCs.

Embodiment 33. The method of embodiment 2 wherein greater than one metabolite is tested to determine intracellular residence time.

Embodiment 34. The method of embodiment 2 wherein the intracellular persistence is determined in PBMCs.

Embodiment 35. The method of embodiment 2 wherein the metabolite comprises the phosphonate group of Metabolite X.

Embodiment 36. The method of embodiment 2 wherein the metabolite comprises an unesterified carboxyl group.

Embodiment 37. The method of embodiment 2 wherein the intracellular depot metabolite comprises the group $-P(O)(OH)-$.

Embodiment 38. A library of candidate non-nucleotide anti-HIV compounds comprising a plurality of candidate compounds suspected to have anti HIV activity which contain esterified carboxyl or esterified phosphonate groups.

Embodiment 39. A library of candidate anti-HIV compounds which does not consist solely of nucleotides and which comprises a plurality of candidate compounds suspected to have anti-HIV activity which contain esterified carboxyl or esterified phosphonate groups.

Embodiment 40. The library of embodiments 38 or 39 comprising at least about 10 candidate compounds.

Embodiment 41. The library of embodiments 38 or 39 wherein the candidate compounds comprise (a) a phosphonate substituted with an amino acid or an organic acid, or (b) an amino acid, at least one of the carboxyl groups of the amino acid or organic acid being esterified.

Embodiment 42. The library of embodiments 38 or 39 wherein the compounds in the library are stored in discrete containers.

Embodiment 43. A method comprising testing the library of embodiments 39, 40, 41, or 42 to determine the anti-HIV activity of at least one candidate compound in the library.

Embodiment 44. The method of embodiment 43 comprising determining for tissue selectivity and/or the intracellular persistence of at least one of said candidate compounds and/or at least one of their intracellular metabolites.

Embodiment 45. The method of embodiment 43 comprising the additional steps of identifying a clinical trial compound from said library, entering into clinical trials with said clinical trial compound, obtaining regulatory approval to market said clinical trial compound for the treatment of HIV, and selling said clinical trial compound after said regulatory approval.

Embodiment 46. Isolated GS-7340 Ester Hydrolase.

Embodiment 47. The Hydrolase of embodiment 46 which is purified to a single major band on gel filtration chromatography.

Embodiment 48. The Hydrolase of embodiment 46 which is capable of being recovered from human PBMC cells.

Embodiment 49. The Hydrolase of embodiment 48 wherein the Hydrolase has a MW on gel filtration chromatography of about 70-100 kDa.

Embodiment 50. The Hydrolase of embodiment 48 which has a pI of about 4.5-5.5 by chromatofocusing.

Embodiment 51. The Hydrolase of embodiment 50 which is inhibited by 3,4 dichloroisocoumarin.

Embodiment 52. The Hydrolase of embodiment 51 which binds to Butyl Sepharose HIC.

Embodiment 53. The Hydrolase of embodiment 52 which binds to anion exchange medium Q15.

Embodiment 54. The Hydrolase of embodiment 53 which binds to hydroxyapatite.

Embodiment 55. The Hydrolase of embodiment 46 which is cross-linked to an insoluble medium.

Embodiment 56. A method comprising obtaining a substantially pure organic molecule, optionally contacting the organic molecule with another molecule to produce a composition, and contacting the Hydrolase of embodiment 46 with said organic molecule or composition.

Embodiment 57. The method of embodiment 56 wherein the organic molecule is an anti-HIV compound.

Embodiment 58. A method comprising contacting GS-7340 Ester Hydrolase with an organic compound in an *in vitro* or cell culture environment.

Embodiment 59. The method of embodiment 58 wherein the environment is cell free.

Embodiment 60. A composition comprising a substantially pure organic compound and isolated GS-7340 Ester Hydrolase.

Embodiment 61. A composition comprising an organic compound and GS-7340 Ester Hydrolase in an *in vitro* or cell culture environment.

Embodiment 62. In a method for identifying an anti-HIV therapeutic compound, the improvement comprising substituting a prototype compound with an esterified phosphonate or esterified carboxyl group to produce a candidate compound and assaying the resulting candidate compound for its anti-HIV activity.

Embodiment 63. The method of embodiment 61 wherein the candidate is assayed for its intracellular persistence.

Embodiment 64. The method of embodiment 63 wherein the candidate is assayed for its extracellular stability against hydrolysis of the carboxyl or phosphonate ester.

Embodiment 65. The method of embodiment 64 comprising selecting from a plurality of candidates a candidate which is esterolytically cleaved intracellularly to yield an intracellular persistent metabolite having anti-HIV activity and which candidate is substantially esterolytically stable against extracellular hydrolysis of the carboxyl or phosphonate ester.

Embodiment 66. The method of embodiment 65 wherein the candidate is substantially stable against hydrolysis of the carboxyl or phosphonate esters outside of lymphoid tissue.

Embodiment 67. The method of embodiment 62 wherein the candidate is substituted with a phosphonate group comprising monosubstitution with (a) an amino acid linked through an amino group to the phosphorus atom or (b) an organic acid, and wherein a carboxylic acid of the amino acid or organic acid is esterified.

Embodiment 68. The method of embodiment 62 wherein the candidate is substituted with a group comprising an amino acid, wherein a carboxylic acid of the amino acid is esterified.

Embodiment 69. The method of embodiment 68 wherein the carboxylic acid is the residue of a hydroxyorganic acid linked to the phosphorus atom through an oxygen atom.

Embodiment 70. The method of embodiments 68 or 69 wherein the hydroxy group of the hydroxyorganic acid or the amino group of the amino acid are in the alpha position.

Embodiment 71. A method for identifying a candidate compound as a suitable pro-drug, comprising:

(a) providing the candidate compound having an esterified phosphonate group or an esterified carboxyl group;

(b) contacting the candidate compound with an extract capable of catalyzing the hydrolysis of a carboxylic ester; and

(c) identifying the candidate compound as a suitable pro-drug if the metabolite compound has a phosphonic acid group instead of the esterified phosphonate group of the candidate compound, or a carboxylic acid group instead of the esterified carboxyl group of the candidate compound.

Embodiment 72. The method of embodiment 71, wherein said extract is obtained from peripheral blood mononuclear cells.

Embodiment 73. A method for identifying a candidate compound as a suitable pro-drug, comprising:

(a) providing the candidate compound having an esterified phosphonate group or an esterified carboxyl group;

(b) contacting the candidate compound with an extract of peripheral blood mononuclear cells having carboxylic ester hydrolase activity to produce a metabolite compound; and

(c) identifying the candidate compound as a suitable pro-drug if the metabolite compound has a phosphonic acid group instead of the esterified phosphonate group of the candidate compound, or a carboxylic acid group instead of the esterified carboxyl group of the candidate compound.

Embodiment 74. The method of embodiment 73, wherein said providing step comprises providing a candidate compound formed by substituting a prototype compound known to have anti-HIV therapeutic activity with an esterified phosphonate or carboxyl group.

Embodiment 75. The method of embodiment 74, wherein said prototype compound is not a nucleoside, and does not contain a nucleoside base.

Embodiment 76. The method of embodiment 73, wherein said providing step comprises providing a candidate compound that is an amino acid phosphonoamidate, wherein a carboxyl group of the amino acid is esterified.

Embodiment 77. The method of embodiment 73, wherein said providing step comprises providing a candidate compound that is substantially stable against extracellular hydrolysis of the esterified group.

Embodiment 78. The method of embodiment 73, wherein said providing step comprises providing a candidate compound formed by substituting a prototype compound.

Embodiment 79. The method of embodiment 73, further comprising (d) determining the intracellular persistence of the candidate compound.

Embodiment 80. The method of embodiment 73, further comprising (d) determining the intracellular persistence of the metabolite compound.

Embodiment 81. The method of embodiment 73, further comprising (d) determining the intracellular persistence of the candidate compound and the metabolite compound.

Embodiment 82. The method of embodiment 73, further comprising (d) determining the tissue selectivity of the candidate compound.

Embodiment 83. The method of embodiment 73, further comprising (d) determining the tissue selectivity of the metabolite compound.

Embodiment 84. The method of embodiment 73, further comprising (d) determining the tissue selectivity of the candidate compound and the metabolite compound.

Embodiment 85. The method of embodiment 73, further comprising (d) determining the anti-HIV protease activity of the metabolite compound.

Embodiment 86. The method of embodiment 73, further comprising (d) determining the HIV-inhibition ability of the candidate compound.

Embodiment 87. The method of embodiment 73, further comprising (d) determining the resistance of HIV to the candidate compound.

Embodiment 88. The method of embodiment 73, further comprising (d) determining the resistance of HIV to the metabolite compound.

Embodiment 89. The method of embodiment 73, further comprising (d) determining the resistance of HIV to the candidate compound and the metabolite compound.

Embodiment 90. The method of embodiment 73, further comprising (d) determining the intracellular residence time of the candidate compound.

Embodiment 91. The method of embodiment 73, further comprising (d) determining the intracellular residence time of the metabolite compound.

Embodiment 92. The method of embodiment 73, further comprising (d) determining the intracellular residence time of the candidate compound and the metabolite compound.

Embodiment 93. The method of embodiment 90, wherein said step of determining the intracellular residence time of the metabolite compound comprises determining the half-life of the metabolite compound within lymphoid tissue.

Embodiment 94. The method of embodiment 91, wherein said step of determining the intracellular residence time of the metabolite compound comprises determining the half-life of the metabolite compound within lymphoid tissue.

Embodiment 95. The method of embodiment 92, wherein said step of determining the intracellular residence time of the metabolite compound comprises determining the half-life of the metabolite compound within lymphoid tissue.

Embodiment 96. The method of embodiment 93, wherein said step of determining the half-life of the metabolite compound further comprises determining the half-life of the metabolite compound within helper cells, killer cells, lymph nodes, or peripheral blood mononuclear cells.

Embodiment 97. The method of embodiment 94, wherein said step of determining the half-life of the metabolite compound further comprises determining the half-life of the metabolite compound within helper cells, killer cells, lymph nodes, or peripheral blood mononuclear cells.

Embodiment 98. The method of embodiment 95, wherein said step of determining the half-life of the metabolite compound further comprises determining the half-life of the metabolite compound within helper cells, killer cells, lymph nodes, or peripheral blood mononuclear cells.

Embodiment 99. The method of embodiment 73, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in a cell-free environment.

Embodiment 100. The method of embodiment 73, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in vitro.

Embodiment 101. The method of embodiment 73, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in cell culture.

Embodiment 102. The method of embodiment 101, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in a culture of peripheral blood mononuclear cells.

Embodiment 103. A method for identifying a candidate compound as a suitable pro-drug, comprising:

- (a) providing the candidate compound having an esterified phosphonate group;
- (b) contacting the candidate compound with GS-7340 Ester Hydrolase to produce a metabolite compound; and
- (c) identifying the candidate compound as a suitable pro-drug if the metabolite compound has a phosphonic acid group instead of the esterified phosphonate group of the candidate compound.

Embodiment 104. The method of embodiment 103, wherein said providing step further comprises monosubstitution of the esterified phosphonate group with an organic acid having an esterified carboxyl group.

Embodiment 105. The method of embodiment 103, wherein said providing step further comprises monosubstitution of the esterified phosphonate group with an amino acid linked through an amino group to the phosphorus atom, wherein the amino acid has an esterified carboxyl group.

Embodiment 106. The method of embodiment 103, wherein said providing step comprises providing a candidate compound formed by substituting a prototype compound known to have anti-HIV therapeutic activity with an esterified phosphonate or carboxyl group.

Embodiment 107. The method of embodiment 106, wherein said prototype compound is not a nucleoside, and does not contain a nucleoside base.

Embodiment 108. The method of embodiment 103, wherein said providing step comprises providing a candidate compound that is an amino acid phosphonoamidate, wherein a carboxyl group of the amino acid is esterified.

Embodiment 109. The method of embodiment 103, wherein said providing step comprises providing a candidate compound that is substantially stable against extracellular hydrolysis of the esterified group.

Embodiment 110. The method of embodiment 103, wherein said providing step comprises providing a candidate compound formed by substituting a prototype compound.

Embodiment 111. The method of embodiment 103, further comprising (d) determining the intracellular persistence of the candidate compound.

Embodiment 112. The method of embodiment 103, further comprising (d) determining the intracellular persistence of the metabolite compound.

Embodiment 113. The method of embodiment 103, further comprising (d) determining the intracellular persistence of the candidate compound and the metabolite compound.

Embodiment 114. The method of embodiment 103, further comprising (d) determining the tissue selectivity of the candidate compound.

Embodiment 115. The method of embodiment 103, further comprising (d) determining the tissue selectivity of the metabolite compound.

Embodiment 116. The method of embodiment 103, further comprising (d) determining the tissue selectivity of the candidate compound and the metabolite compound.

Embodiment 117. The method of embodiment 103, further comprising (d) determining the anti-HIV protease activity of the metabolite compound.

Embodiment 118. The method of embodiment 103, further comprising (d) determining the HIV-inhibition ability of the candidate compound.

Embodiment 119. The method of embodiment 103, further comprising (d) determining the resistance of HIV to the candidate compound.

Embodiment 120. The method of embodiment 103, further comprising (d) determining the resistance of HIV to the metabolite compound.

Embodiment 121. The method of embodiment 103, further comprising (d) determining the resistance of HIV to the candidate compound and the metabolite compound.

Embodiment 122. The method of embodiment 103, further comprising (d) determining the intracellular residence time of the candidate compound.

Embodiment 123. The method of embodiment 103, further comprising (d) determining the intracellular residence time of the metabolite compound.

Embodiment 124. The method of embodiment 103, further comprising (d) determining the intracellular residence time of the candidate compound and the metabolite compound.

Embodiment 125. The method of embodiment 122, wherein said step of determining the intracellular residence time of the metabolite compound comprises determining the half-life of the metabolite compound within lymphoid tissue.

Embodiment 126. The method of embodiment 123, wherein said step of determining the intracellular residence time of the metabolite compound comprises determining the half-life of the metabolite compound within lymphoid tissue.

Embodiment 127. The method of embodiment 124, wherein said step of determining the intracellular residence time of the metabolite compound comprises determining the half-life of the metabolite compound within lymphoid tissue.

Embodiment 128. The method of embodiment 125, wherein said step of determining the half-life of the metabolite compound further comprises determining the half-life of the metabolite compound within helper cells, killer cells, lymph nodes, or peripheral blood mononuclear cells.

Embodiment 129. The method of embodiment 126, wherein said step of determining the half-life of the metabolite compound further comprises determining the half-life of the metabolite compound within helper cells, killer cells, lymph nodes, or peripheral blood mononuclear cells.

Embodiment 130. The method of embodiment 127, wherein said step of determining the half-life of the metabolite compound further comprises determining the half-life of the metabolite compound within helper cells, killer cells, lymph nodes, or peripheral blood mononuclear cells.

Embodiment 131. The method of embodiment 103, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in a cell-free environment.

Embodiment 132. The method of embodiment 103, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in vitro.

Embodiment 133. The method of embodiment 103, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in cell culture.

Embodiment 134. The method of embodiment 133, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in a culture of peripheral blood mononuclear cells.

Embodiment 135. A method for identifying a candidate compound as a suitable pro-drug, comprising:

- (a) providing the candidate compound having an esterified carboxyl group;

(b) contacting the candidate compound with GS-7340 Ester Hydrolase to produce a metabolite compound; and

(c) identifying the candidate compound as a suitable pro-drug if the metabolite compound has a carboxylic acid group instead of the esterified carboxyl group of the candidate compound.

Embodiment 136. The method of embodiment 135, wherein said providing step comprises providing a candidate compound substituted with an amino acid group, wherein the amino acid has an esterified carboxyl group.

Embodiment 137. The method of embodiment 135, wherein said providing step comprises providing a candidate compound formed by substituting a prototype compound known to have anti-HIV therapeutic activity with an esterified phosphonate or carboxyl group.

Embodiment 138. The method of embodiment 137, wherein said prototype compound is not a nucleoside, and does not contain a nucleoside base.

Embodiment 139. The method of embodiment 135, wherein said providing step comprises providing a candidate compound that is an amino acid phosphonoamidate, wherein a carboxyl group of the amino acid is esterified.

Embodiment 140. The method of embodiment 135, wherein said providing step comprises providing a candidate compound that is substantially stable against extracellular hydrolysis of the esterified group.

Embodiment 141. The method of embodiment 135, wherein said providing step comprises providing a candidate compound formed by substituting a prototype compound.

Embodiment 142. The method of embodiment 135, further comprising (d) determining the intracellular persistence of the candidate compound.

Embodiment 143. The method of embodiment 135, further comprising (d) determining the intracellular persistence of the metabolite compound.

Embodiment 144. The method of embodiment 135, further comprising (d) determining the intracellular persistence of the candidate compound and the metabolite compound.

Embodiment 145. The method of embodiment 135, further comprising (d) determining the tissue selectivity of the candidate compound.

Embodiment 146. The method of embodiment 135, further comprising (d) determining the tissue selectivity of the metabolite compound.

Embodiment 147. The method of embodiment 135, further comprising (d) determining the tissue selectivity of the candidate compound and the metabolite compound.

Embodiment 148. The method of embodiment 135, further comprising (d) determining the anti-HIV protease activity of the metabolite compound.

Embodiment 149. The method of embodiment 135, further comprising (d) determining the HIV-inhibition ability of the candidate compound.

Embodiment 150. The method of embodiment 135, further comprising (d) determining the resistance of HIV to the candidate compound.

Embodiment 151. The method of embodiment 135, further comprising (d) determining the resistance of HIV to the metabolite compound.

Embodiment 152. The method of embodiment 135, further comprising (d) determining the resistance of HIV to the candidate compound and the metabolite compound.

Embodiment 153. The method of embodiment 135, further comprising (d) determining the intracellular residence time of the candidate compound.

Embodiment 154. The method of embodiment 135, further comprising (d) determining the intracellular residence time of the metabolite compound.

Embodiment 155. The method of embodiment 135, further comprising (d) determining the intracellular residence time of the candidate compound and the metabolite compound.

Embodiment 156. The method of embodiment 153, wherein said step of determining the intracellular residence time of the metabolite compound comprises determining the half-life of the metabolite compound within lymphoid tissue.

Embodiment 157. The method of embodiment 154, wherein said step of determining the intracellular residence time of the metabolite compound comprises determining the half-life of the metabolite compound within lymphoid tissue.

Embodiment 158. The method of embodiment 155, wherein said step of determining the intracellular residence time of the metabolite compound comprises determining the half-life of the metabolite compound within lymphoid tissue.

Embodiment 159. The method of embodiment 156, wherein said step of determining the half-life of the metabolite compound further comprises determining the half-life of the metabolite compound within helper cells, killer cells, lymph nodes, or peripheral blood mononuclear cells.

Embodiment 160. The method of embodiment 157, wherein said step of determining the half-life of the metabolite compound further comprises determining the half-life of the metabolite compound within helper cells, killer cells, lymph nodes, or peripheral blood mononuclear cells.

Embodiment 161. The method of embodiment 158, wherein said step of determining the half-life of the metabolite compound further comprises determining the half-life of the

metabolite compound within helper cells, killer cells, lymph nodes, or peripheral blood mononuclear cells.

Embodiment 162. The method of embodiment 135, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in a cell-free environment.

Embodiment 163. The method of embodiment 135, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in vitro.

Embodiment 164. The method of embodiment 135, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in cell culture.

Embodiment 165. The method of embodiment 164, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in a culture of peripheral blood mononuclear cells.

Embodiment 166. A method for identifying a candidate compound as a suitable pro-drug, comprising:

(a) providing the candidate compound having an esterified phosphonate group or an esterified carboxyl group;

(b) contacting the candidate compound with an extract of peripheral blood mononuclear cells which has carboxylic ester hydrolase activity but does not cleave alpha-naphthyl acetate, to produce a metabolite compound; and

(c) identifying the candidate compound as a suitable pro-drug if the metabolite compound has a phosphonic acid group instead of the esterified phosphonate group of the candidate compound, or a carboxylic acid group instead of the esterified carboxyl group of the candidate compound.

Embodiment 167. The method of embodiment 166, wherein said providing step comprises providing a candidate compound formed by substituting a prototype compound known to have anti-HIV therapeutic activity with an esterified phosphonate or carboxyl group.

Embodiment 168. The method of embodiment 167, wherein said prototype compound is not a nucleoside, and does not contain a nucleoside base.

Embodiment 169. The method of embodiment 166, wherein said providing step comprises providing a candidate compound that is an amino acid phosphonoamidate, wherein a carboxyl group of the amino acid is esterified.

Embodiment 170. The method of embodiment 166, wherein said providing step comprises providing a candidate compound that is substantially stable against extracellular hydrolysis of the esterified group.

Embodiment 171. The method of embodiment 166, wherein said providing step comprises providing a candidate compound formed by substituting a prototype compound.

Embodiment 172. The method of embodiment 166, further comprising (d) determining the intracellular persistence of the candidate compound.

Embodiment 173. The method of embodiment 166, further comprising (d) determining the intracellular persistence of the metabolite compound.

Embodiment 174. The method of embodiment 166, further comprising (d) determining the intracellular persistence of the candidate compound and the metabolite compound.

Embodiment 175. The method of embodiment 166, further comprising (d) determining the tissue selectivity of the candidate compound.

Embodiment 176. The method of embodiment 166, further comprising (d) determining the tissue selectivity of the metabolite compound

Embodiment 177. The method of embodiment 166, further comprising (d) determining the tissue selectivity of the candidate compound and the metabolite compound.

Embodiment 178. The method of embodiment 166, further comprising (d) determining the anti-HIV protease activity of the metabolite compound.

Embodiment 179. The method of embodiment 166, further comprising (d) determining the HIV-inhibition ability of the candidate compound.

Embodiment 180. The method of embodiment 166, further comprising (d) determining the resistance of HIV to the candidate compound.

Embodiment 181. The method of embodiment 166, further comprising (d) determining the resistance of HIV to the metabolite compound.

Embodiment 182. The method of embodiment 166, further comprising (d) determining the resistance of HIV to the candidate compound and the metabolite compound.

Embodiment 183. The method of embodiment 166, further comprising (d) determining the intracellular residence time of the candidate compound.

Embodiment 184. The method of embodiment 166, further comprising (d) determining the intracellular residence time of the metabolite compound.

Embodiment 185. The method of embodiment 166, further comprising (d) determining the intracellular residence time of the candidate compound and the metabolite compound.

Embodiment 186. The method of embodiment 183, wherein said step of determining the intracellular residence time of the metabolite compound comprises determining the half-life of the metabolite compound within lymphoid tissue.

Embodiment 187. The method of embodiment 184, wherein said step of determining the intracellular residence time of the metabolite compound comprises determining the half-life of the metabolite compound within lymphoid tissue.

Embodiment 188. The method of embodiment 185, wherein said step of determining the intracellular residence time of the metabolite compound comprises determining the half-life of the metabolite compound within lymphoid tissue.

Embodiment 189. The method of embodiment 186, wherein said step of determining the half-life of the metabolite compound further comprises determining the half-life of the metabolite compound within helper cells, killer cells, lymph nodes, or peripheral blood mononuclear cells.

Embodiment 190. The method of embodiment 187, wherein said step of determining the half-life of the metabolite compound further comprises determining the half-life of the metabolite compound within helper cells, killer cells, lymph nodes, or peripheral blood mononuclear cells.

Embodiment 191. The method of embodiment 188, wherein said step of determining the half-life of the metabolite compound further comprises determining the half-life of the metabolite compound within helper cells, killer cells, lymph nodes, or peripheral blood mononuclear cells.

Embodiment 192. The method of embodiment 166, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in a cell-free environment.

Embodiment 193. The method of embodiment 166, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in vitro.

Embodiment 194. The method of embodiment 166, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in cell culture.

Embodiment 195. The method of embodiment 194, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in a culture of peripheral blood mononuclear cells.

Embodiment 196. A candidate compound identified by the method of embodiment 71, wherein the candidate compound is an amino acid phosphonoamidate in which a carboxyl group of the amino acid is esterified.

Embodiment 197. A candidate compound identified by the method of embodiment 103, wherein the candidate compound is an amino acid phosphonoamidate in which a carboxyl group of the amino acid is esterified.

Embodiment 198. A candidate compound identified by the method of embodiment 134, wherein the candidate compound is an amino acid phosphonoamidate in which a carboxyl group of the amino acid is esterified.

Embodiment 199. A candidate compound identified by the method of embodiment 164, wherein the candidate compound is an amino acid phosphonoamidate in which a carboxyl group of the amino acid is esterified.

Embodiment 200. A candidate compound identified by the method of embodiment 71, wherein the candidate compound is substituted with an amino acid group in which a carboxyl group of the amino acid is esterified.

Embodiment 201. A candidate compound identified by the method of embodiment 103, wherein the candidate compound is substituted with an amino acid group in which a carboxyl group of the amino acid is esterified.

Embodiment 202. A candidate compound identified by the method of embodiment 134, wherein the candidate compound is substituted with an amino acid group in which a carboxyl group of the amino acid is esterified.

Embodiment 203. A candidate compound identified by the method of embodiment 164, wherein the candidate compound is substituted with an amino acid group in which a carboxyl group of the amino acid is esterified.

Embodiment 204. The candidate compound of embodiment 200, wherein the amino group of the amino acid is in the alpha position.

Embodiment 205. The candidate compound of embodiment 201, wherein the amino group of the amino acid is in the alpha position.

Embodiment 206. The candidate compound of embodiment 202, wherein the amino group of the amino acid is in the alpha position.

Embodiment 207. The candidate compound of embodiment 203, wherein the amino group of the amino acid is in the alpha position.

Embodiment 208. A candidate compound identified by the method of embodiment 71, wherein the esterified phosphonate group is monosubstituted with a hydroxyorganic acid linked to the phosphorus atom through an oxygen atom.

Embodiment 209. The candidate compound of embodiment 133, wherein the hydroxy group of the hydroxyorganic acid is in the alpha position.

Embodiment 210. A candidate compound identified by the method of embodiment 71, wherein the candidate compound is substantially stable against extracellular hydrolysis of the esterified group.

Embodiment 211. A candidate compound identified by the method of embodiment 103, wherein the candidate compound is substantially stable against extracellular hydrolysis of the esterified group.

Embodiment 212. A candidate compound identified by the method of embodiment 134, wherein the candidate compound is substantially stable against extracellular hydrolysis of the esterified group.

Embodiment 213. A candidate compound identified by the method of embodiment 164, wherein the candidate compound is substantially stable against extracellular hydrolysis of the esterified group.

Embodiment 214. A method of screening candidate compounds for suitability as anti-HIV therapeutic agents, comprising:

- (a) providing a candidate compound identified by the method of embodiment 71;
- (b) determining the anti-HIV activity of the candidate compound; and
- (c) determining the intracellular persistence of the candidate compound.

Embodiment 215. A method of screening candidate compounds for suitability as anti-HIV therapeutic agents, comprising:

- (a) providing a candidate compound identified by the method of embodiment 103;
- (b) determining the anti-HIV activity of the candidate compound; and
- (c) determining the intracellular persistence of the candidate compound.

Embodiment 216. A method of screening candidate compounds for suitability as anti-HIV therapeutic agents, comprising:

- (a) providing a candidate compound identified by the method of embodiment 134;
- (b) determining the anti-HIV activity of the candidate compound; and

- (c) determining the intracellular persistence of the candidate compound.

Embodiment 217. A method of screening candidate compounds for suitability as anti-HIV therapeutic agents, comprising:

- (a) providing a candidate compound identified by the method of embodiment 164;
- (b) determining the anti-HIV activity of the candidate compound; and
- (c) determining the intracellular persistence of the candidate compound.

Embodiment 218. The method of embodiment 214, wherein said step (b) comprises determining the activity of the candidate compound against HIV protease.

Embodiment 219. The method of embodiment 215, wherein said step (b) comprises determining the activity of the candidate compound against HIV protease.

Embodiment 220. The method of embodiment 216, wherein said step (b) comprises determining the activity of the candidate compound against HIV protease.

Embodiment 221. The method of embodiment 217, wherein said step (b) comprises determining the activity of the candidate compound against HIV protease.

Embodiment 222. The method of embodiment 214, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV.

Embodiment 223. The method of embodiment 215, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV.

Embodiment 224. The method of embodiment 216, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV.

Embodiment 225. The method of embodiment 217, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV.

Embodiment 226. The method of embodiment 222, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV protease.

Embodiment 227. The method of embodiment 223, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV protease.

Embodiment 228. The method of embodiment 224, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV protease.

Embodiment 229. The method of embodiment 225, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV protease.

Embodiment 230. The method of embodiment 222, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV integrase.

Embodiment 231. The method of embodiment 223, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV integrase.

Embodiment 232. The method of embodiment 224, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV integrase.

Embodiment 233. The method of embodiment 225, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV integrase.

Embodiment 234. The method of embodiment 222, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV reverse transcriptase.

Embodiment 235. The method of embodiment 223, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV reverse transcriptase.

Embodiment 236. The method of embodiment 224, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV reverse transcriptase.

Embodiment 237. The method of embodiment 225, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV reverse transcriptase.

Embodiment 238. The method of embodiment 214, wherein said step (b) further comprises determining the resistance of HIV to the candidate compound.

Embodiment 239. The method of embodiment 214, wherein said step (b) is performed by *in vitro* assay.

Embodiment 240. The method of embodiment 214, wherein said step (b) further comprises determining the anti-HIV activity of an acid metabolite of the candidate compound.

Embodiment 241. The method of embodiment 240, wherein said acid metabolite is a carboxylic acid compound formed by esterolytic hydrolysis of the candidate compound.

Embodiment 242. The method of embodiment 240, wherein said acid metabolite is a phosphonic acid compound formed by esterolytic hydrolysis of the candidate compound.

Embodiment 243. The method of embodiment 214, wherein said step (c) comprises determining the intracellular residence time of the candidate compound.

Embodiment 244. The method of embodiment 214, wherein said step (c) further comprises determining the intracellular residence time of an acid metabolite of the candidate compound.

Embodiment 245. The method of embodiment 244, wherein said acid metabolite is a carboxylic acid compound formed by esterolytic hydrolysis of the candidate compound.

Embodiment 246. The method of embodiment 244, wherein said acid metabolite is a phosphonic acid compound formed by esterolytic hydrolysis of the candidate compound.

Embodiment 247. The method of embodiment 244, wherein said step (c) further comprises determining the half-life of the metabolite compound within lymphoid tissue.

Embodiment 248. The method of embodiment 247, wherein in said step of determining the half-life of the metabolite compound within lymphoid tissue, the lymphoid tissue is selected from the group consisting of helper cells, killer cells, lymph nodes, and peripheral blood mononuclear cells.

Embodiment 249. The method of embodiment 214, further comprising (d) determining the tissue selectivity of the candidate compound.

Embodiment 250. The method of embodiment 249, wherein said step (d) further comprises determining the tissue selectivity of an acid metabolite of the candidate compound.